

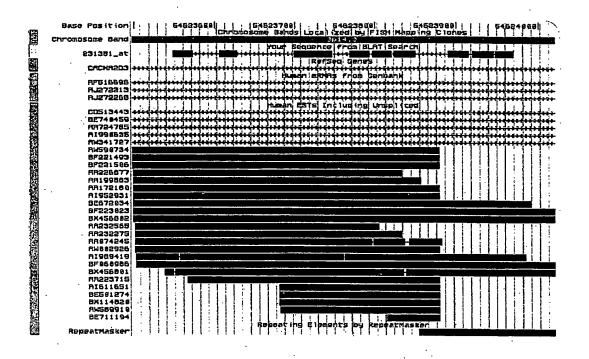
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| 7 |
|--------|
| Figure |

| MOTIFS | homeobox | nuclear localization and leucine zipper domalns | | 'cold-shock' DNA-binding and zinc finger domains | C2H2 zinc finger domains | |
|---------------------------------------|------------|--|--------------|---|--------------------------|------|
| MOUSE ORTHOLOGUES | Pouf1/Oct4 | ī | ŧ | Tex17 | ı | |
| NORMALIZED ABUNDANCE IN GERM CELLS | 76% | 78% | %56 . | %99 | %85 | |
| ES (±SE) | 1228±107 | 1096±30 | 625±187 | 1319±81 | 15∓299 | |
| . ES/EB | 154 | 519 | . 22 | 31 | 27 | |
| RT-PCR . | | | | | | |
| GENE | Hs.2860 | Hs.67624 | Hs.351262 | Hs.86154 | Hs.189095 | ВРОН |

Summary:

The Affymetrix probeset 231381_at. exhibited an ES/EB ratio of 385. This probeset was derived from the Human UniGene cluster Hs.67624. This cluster is composed of 24 individual EST sequences most of which are derived from ES cell lines and germ cell tumors. It is also represented by the Genbank sequence BF223023. This mRNA appears to be encoded within the intron of another gene, the voltage-gated calcium channel alpha(2)delta-3 subunit gene. This mRNA appears to be transcribed from an endogenous human retrovirus which has been spliced to a non-viral sequence at its 3' end. This 3' sequence is unique in the genome. This mRNA does not appear to encode a complete protein. The 3' exon of the mRNA shares similarity with the Rat low density lipoprotein receptor-related protein 2 however this exon contains stop codons suggesting that it may represent a transcript derived from a pseudogene.



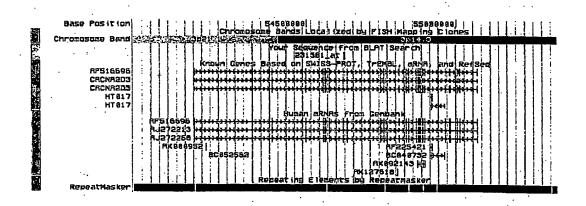
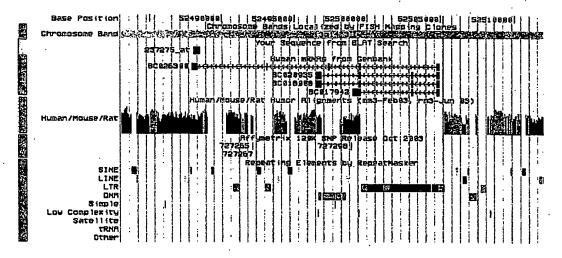


Figure 3

The Affymetrix probeset 237275_at exhibited an ES/EB ratio of 39. This probeset was derived from the Human Unigene cluster Hs.143925 which is also represented by the Genbank clone BC026300. This mRNA is composed of five exons and is encoded by a gene located on Chromosome 13. This gene appears to encode three different related mRNAs represented by BC026300, BC018008 and BC017942 as displayed below, but the 237275_at probeset is specific for the 3' exon of BC026300.

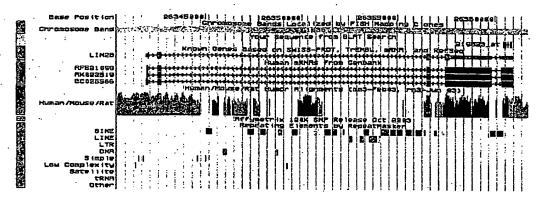


The BC026300 mRNA has three exons that contain repetitive DNA sequences and two exons that contains unique DNA sequences that have been conserved across the mouse, rat and human genomes.

Protein:

The longest open reading within this mRNA is located in the 3' exon containing unique sequences. The amino acid sequence of this open reading frame does is not similar to any known protein. Comparison of this peptide to the Pfam database http://pfam.wustl.edu/ using hmmsearch reveals no similarity to know protein motifs. Analysis of this coding sequence with the methods of Fickett (NAR 10(17); 5303-5318 (1982)) and Gribskov et al. (NAR 12(1); 539-549 (1984)) as implemented in The GCG Wisconsin Package applications Testcode and CodonPreference suggest that this sequence may not actually encode protein.

The Affymetrix probeset 219823_at exhibited an ES/EB ratio of 26. This probeset was derived from the Human Unigene cluster Hs.86154 which is also represented by the RefSeq clone NM_024674. This mRNA encodes a 209 amino acid protein (below) that is similar to the c. elegans lin-28 RNA-binding protein.



Protein Motifs:

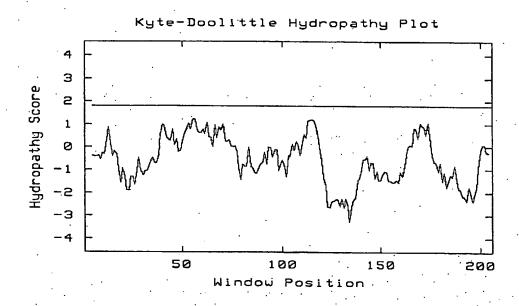
Comparison of the protein derived from this sequence to the Pfam database http://pfam.wustl.edu/ using hmmsearch reveals the presence of a Cold-shock DNA-binding domain sequence and Zinc knuckle domain sequence as diagramed below. The presence of these sequences suggest that this protein is involved in binding RNA or single stranded DNA.



Hydropathy:

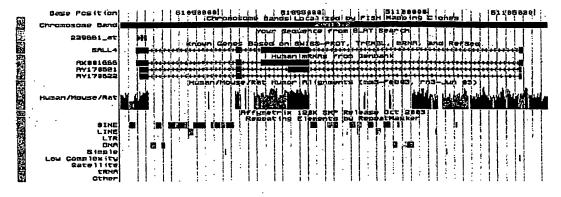
Kyte-Doolittle Hydropathy Plot (window size = 9)

A hydropathy plot of this protein indicates that is a relatively hydrophilic protein with no transmembrane domains (see below).



Figur 5

The Affymetrix probeset 229661_at exhibited an ES/EB ratio of 23. This probeset was derived from the Human Unigene cluster Hs.189095 which is also represented by the RefSeq clone NM_020436. This mRNA is encoded by a gene located on Chromosome 20 that has been genetically linked to the congenital opthalmoplegia 'Duane radial ray syndrome'. This mRNA encodes a 1053 amino acid protein known as Sall4 that is a member of a family of proteins that contain a C2H2 zinc finger domain and are thought to be transcription factors.



Protein Motifs:

Comparison of the protein derived from this sequence to the Pfam database http://pfam.wustl.edu/ using hmmsearch reveals the presence of seven C2H2 zinc finger domains as diagramed:



Hydropathy:

Kyte-Doolittle Hydropathy Plot (window size = 9)

A hydropathy plot of this protein indicates that is a relatively hydrophilic protein with no transmembrane domains (see below).

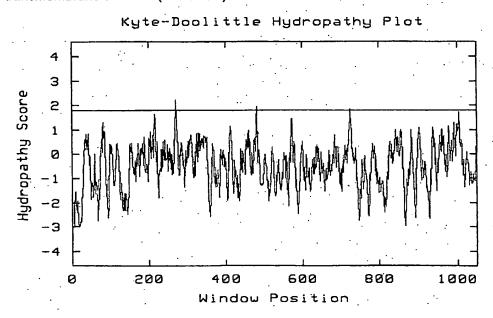
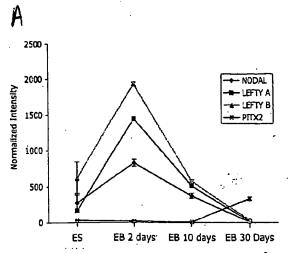


Figure 6



NODAL
LEFTY A
LEFTY B
PITX2
GAPDH

Figure 7

| Genbank M14087.1 BF223023 NM_002701.1 AF020589.1 AF193855.1 NM_024865.1 AC005378 NM_001100.2 NM_001898.1 NM_011808.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01898.1 NM_01845.1 AI659815 AL137654.1 NM_013445.1 BG540454 AI669815 AN137654.1 NM_013445.1 NM_017488.1 NM_017488.1 NM_006892.1 AI050866 | NM_003413.1 NM_003504.1 BE779765 NM_000810.2 NM_001255.1 NM_024749.1 BF971587 NM_001813.1 |
|--|--|
| Unigene Hs.287389 Hs.2860 Hs.2860 Hs.132863 J1Hs.326290 Hs.106552 Hs.106552 Hs.123114 j. Hs.333145 Hs.186962 Hs.1907 Hs.184945 Hs.1907 Hs.184945 Hs.1907 Hs.184945 Hs.28635 Hs.2679 Hs.25766 Hs.25766 Hs.25766 Hs.25766 Hs.25766 Hs.25766 Hs.25767 Hs.251673 Hs.251673 Hs.251673 | Hs.30837 Hs.24969 Hs.24966 Hs.125741 Hs.336780 Hs.75573 |
| SP C C C C C C C C C C C C C C C C C C C | Lic ramily member 3 neterotaxy 1 (odd-paired homolog, brosophila) CDC45-like protein Homolog of Drospohila Orthodenticle 2 homeobox transcription factor gamma-aminobutyric acid (GABA) A receptor, alpha 5 CDC20 cell division cycle 20 homolog (S. cerevisiae) hypothetical protein FU12505 tubulin, beta polypeptide centromere protein E (312kD) |
| 755 216405_at 385 231381_at 243 208286_x_at 166 224344_at 166 224344_at 166 224344_at 165 223642_at 113 215145_s_at 110 203872_at 92 220085_at 77 206286_s_at 77 206286_s_at 77 206286_s_at 77 206286_s_at 77 206286_s_at 78 206291_at 56 210560_at 56 210560_at 57 20669_at 48 215509_s_at 44 231407_s_at 44 231407_s_at 41 230356_at 41 230568_s_at 41 23 | |
| ES/EB 2 2 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 | 35 35 35 36 36 36 |

| 37 | 33 204766 e at | nidix (nicleoside dinhosphate linked moisty V_tx, a control | 200 | £ 000 MM | |
|------------|-----------------------|--|--------------|--------------|--|
| , (| | יייייי איייייי איייייי מייייייי איייייייי | 005.50 | NM_002452.1 | |
| χ | 32 208580_X_at | H4 histone family, member E | | NM_021968.1 | |
| 4 | 32 205478_at | protein phosphatase 1, regulatory (inhibitor) subunit 1A | Hs.76780 | NM_006741.1 | |
| 33 | 32 204469_at | protein tyrosine phosphatase, receptor-type, Z polypeptide 1 | Hs.78867 | NM 002851.1 | |
| 41 | 31 227434_at | hypothetical protein DKFZp761D2324 | Hs.7981 | A1972623 | |
| 43 | 29 203976_s_at | chromatin assembly factor 1, subunit A (p150) | Hs.79018 | NM_005483.1 | |
| 45 | 29 212873_at | minor histocompatibility antigen HA-1 | Hs.196914 | BE349017 | |
| 4 | 28 211603_s_at | Ad E1A enhancer-binding protein (ETS-domain transcription factor) | | U35622.2 | |
| 45 | 28 219170_at | fibronectin type 3 and SPRY domain-containing protein | Hs.28144 | NM 024333.1 | |
| 46 | 28 221520_s_at | hypothetical protein FLJ10468 | Hs.48855 | BC001651.1 | |
| 47 | 28 209581_at | similar to rat HREV107 | Hs,37189 | BC001387.1 | |
| 48 | 27 224753_at | no identification; protein product of unknown function | Hs.23044 | BE614410 | |
| 49 | 27 203129_s_at | kinesin family member 5C | Hs:6641 | BF059313 | |
| 25 | 26 241530 <u>.</u> at | human homolog of Aspergillus sudD (suppressor of bimD6, mitotic block Hs. 105168 | cl Hs.105168 | AA814371 | |
| 20 | 26 204775_at | chromatin assembly factor 1, subunit B (p60) | Hs.75238 | NM_005441.1 | |
| 23 | 26 219823_at | hypothetical protein FLJ12457 | Hs.86154 | NM_024674.1 | |
| 21 | 26 221805_at | neurofilament, light polypeptide (68kD) | Hs.211584 | _NM_006158.1 | |
| 22 | 25 201430_s_at | dihydropyrimidinase-like 3 | Hs.74566 | W72516 | |
| 27 | 25 210457_x_at | high-mobility group (nonhistone chromosomal) protein isoforms I and | .>- | AF176039.1 | |
| 26 | 25 219942_at | myosin light chain 2a | Hs.75636 | NM 021223.1 | |
| 28 | 25 217728_at | S100 calcium binding protein A6 (calcyclin) | Hs.275243 | NM_014624.2 | |
| 5 4 | 25 205547_s_at | transgelin | Hs.75777 | NM_003186.2 | |
| 23 | | no identification | Hs.152129 | BF672169 | |
| 62 | 23.209807_s_at | nuclear factor I/X (CCAAT-binding transcription factor) | Hs.35841 | Ú18759.1 | |
| 90 | | similar to SALL1 (sal (Drosophila)-like | Hs.189095 | NM_020436.1 | |
| 61 | 23 205691_at | synaptogyrin 3 | Hs.6467 | NM_004209.2 | |
| 49 | 22 204560_at | FK506-binding protein 5 | | NM_004117.1 | |
| 83 | 22 204696_s_at | cell division cycle 25A | Hs.1634 | NM_001789.1 | |
| 65 | 22 205347_s_at | thymosin, beta, identified in neuroblastoma cells | Hs.56145 | NM_021992.1 | |
| .67 | 21 221051_s_at | muscle-specific beta 1 integrin binding protein | Hs.135458 | NM_014446.1 | |
| 99 | 21 206893_at | sal-like 1 (Drosophila) | Hs.123094 | NM_002968.1 | |
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